U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-265 253

Toxic Effects of Hexavalent Chromium on Brook Trout ('Salvelinus fontinalis') and Rainbow Trout ('Salmo gairdneri')

Environmental Research Lab -Duluth, Minn

1 Dec 75

ERL-Dul-050

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-76-013	2. JOURNAL ARTICLE
3. PB 265 253	
4. TITLE AND SUBTITLE: Toxic Effects of Hexavalent Chromium on Brook Trout (Salvelinus fontinalis) and Rainbow Trout (Salmo gairdneri)*	5. REPORT DATE: 1976
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): D.A. Benoit	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth MN 55804	10. PROGRAM ELEMENT NO. 1BA608
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth MN 55804	13. TYPE OF REPORT AND PERIOD COVERED: Journal Article
	14. SPONSORING AGENCY CODE: EPA/600/03

15. SUPPLEMENTARY NOTES

*as published in Water Research, Vol 10, pp 497-500 (Printed in Great Britain)

16. ABSTRACT

Exposing brook trout to various concentrations of chromium [Cr(VI)] for up to 22 months (including reproduction) significantly increased alevin mortality at 0.35 mg $Cr l^{-1}$ and retarded growth of young brook trout at the lowest concentration tested (0.01 mg $Cr l^{-1}$). Eight month exposures of rainbow trout significantly increased alevin mortality at 0.34 mg $Cr l^{-1}$ and also retarded growth at the lowest concentration tested (0.10 mg $Cr l^{-1}$). Exposures of brook trout lasting 22 months showed, however, that growth was only temporarily affected, and therefore, it was not used as an end point to measure the affects of chromium on either species. Reproduction, and embryo hatchability of brook trout were unaffected at Cr(VI) concentrations that affected survival of newly hatched alevins. The maximum acceptable toxicant concentration (MATC) for brook and rainbow trout exposed to Cr(VI) in water with a hardness of 45 mg l^{-1} (as $CaCO_3$) and a pH range of 7-8 lies between 0.20 and 0.35 mg $Cr l^{-1}$. The 96-h LC50 for brook and rainbow trout was 59 and 69 mg $Cr l^{-1}$, respectively; therefore, the application factor (MATC/96-h LC50) for both species lies between 0.003 and 0.006.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chromium Fishes Toxicology Water Pollution	Brook trout Rainbow trout Chronic	06F 06T

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: Release to public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES: 6
	20. SECURITY CLASS (This page): Unclassified	22. PRICE: PC-A02/MF-A01

EPA Form 2220-1 (9-73)

EPA-600/J-76-013
JOURNAL ARTICLE

Water Research Vol. 10, pp. 497 to 500. Pergamon Press 1976. Printed in Great Britain.

TOXIC EFFECTS OF HEXAVALENT CHROMIUM ON BROOK TROUT (*SALVELINUS FONTINALIS*) AND RAINBOW TROUT (*SALMO GAIRDNERI*)

D. A. BENOIT

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804, U.S.A.

(*Received* 1 *December* 1975)

Abstract—Exposing brook trout to various concentrations of chromium [Cr(VI)] for up to 22 months (including reproduction) significantly increased alevin mortality at 0.35 mg Cr l^{-1} and retarded growth of young brook trout at the lowest concentration tested (0.01 mg Cr l^{-1}). Eight month exposures of rainbow trout significantly increased alevin mortality at 0.34 mg Cr l^{-1} and also retarded growth at the lowest concentration tested (0.10 mg Cr l^{-1}). Exposures of brook trout lasting 22 months showed, however, that growth was only temporarily affected, and therefore, it was not used as an end point to measure the affects of chromium on either species. Reproduction, and embryo hatchability of brook trout were unaffected at Cr(VI) concentrations that affected survival of newly hatched alevins. The maximum acceptable toxicant concentration (MATC) for brook and rainbow trout exposed to Cr(VI) in water with a hardness of 45 mg l^{-1} (as $CaCO_3$) and a pH range of 7–8 lies between 0.20 and 0.35 mg Cr l^{-1}. The 96-h LC_{50} for brook and rainbow trout was 59 and 69 mg Cr l^{-1} respectively; therefore, the application factor (MATC/96-h LC_{50}) for both species lies between 0.003 and 0.006.

INTRODUCTION

Information on the chronic toxicity of hexavalent chromium [Cr(VI)] to salmonids is not available except for the work of Olson and Foster (1956), who showed growth effects on early life history stages (embryo through 4-month-old juvenile) of chinook salmon (*Oncorhynchus ishawytscha*) and rainbow trout (*Salmo gairdneri*) at 0.02 mg Cr l^{-1}. This value was considered low when compared to the 96-h LC_{50} of 17.6, 118.0, and 30.0 mg Cr l^{-1} for fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), and guppies (*Poecilia reticulata*), respectively (Pickering and Henderson, 1966). The present study, therefore, was initiated to extend Olson and Foster's work with rainbow trout and to determine the chronic toxic effect of Cr(VI) on brook trout (*Salvelinus fontinalis*) survival, growth, and reproduction.

The first phase of this exposure was designed to investigate chromium's long-term effects on survival and growth of brook trout and rainbow trout (alevin through 8-month-old juvenile). The second phase involved a complete generation toxicant exposure of brook trout (alevin through adult) to determine if the reproductive capacity of the trout was a more sensitive indicator of Cr(VI) toxicity than the early life history stages. The chronic brook trout exposure also allowed the determination of a maximum acceptable toxicant concentration (MATC), for which procedures described by Mount and Stephan (1967) were used.

MATERIALS AND METHODS

The exposure of brook trout and rainbow trout to Cr(VI) was divided into three separate experiments as follows: an 8-month brook trout exposure (embryo through juvenile); an 8-month rainbow trout exposure (alevin through juvenile); and a 22-month brook trout exposure (alevin through adult), which included reproduction and a 3-month exposure of offspring.

Physical

The basic exposure system used for all three tests was similar to that described by McKim and Benoit (1971). A 1-l. proportional diluter (Mount and Brungs, 1967) with a 0.5 dilution factor was used to maintain various concentrations of Cr(VI) during each test. Flow rates throughout the three tests averaged four tank water volumes in 24 h. Quarterly mean water temperatures (°C) during each test were: March, 7°C; June, 12°C; September, 15°C; and December, 9°C.

Eight-month brook trout test. Lake Superior water was obtained from the Duluth city water supply system, which was dechlorinated by passing it through a series of three carbon filters. Sodium thiosulfate (0.1–0.2 mg l^{-1}) was also added to aid in reducing the chloramine residual to ≤ 0.001 mg l^{-1}. Five concentrations of Cr(VI) ranging from 0.01 to 0.20 mg Cr l^{-1} plus one control were maintained throughout the 8-month exposure.

Twentytwo-month brook trout test. This experiment was conducted in the same exposure system used during the 8-month brook trout test. Water was obtained directly from Lake Superior throughout the exposure and temperatures were maintained at a constant 9°C during spawning, embryo incubation, and the 3-month alevin–juvenile growth study. Five concentrations of Cr(VI) ranging from 0.35 to 6.37 mg Cr l^{-1} plus one control were maintained during the first 3-months of the test, after which only the control and lower three concentrations were maintained (0.35, 0.76, and 1.56 mg Cr l^{-1}) because all fish had died at 3.10 and 6.37 mg Cr(VI).

Eight-month rainbow trout test. This exposure was started 3 months after the 22 month brook trout test, and was conducted in the same exposure system. Six exposure tanks were added to the system and each concentration divided equally from the diluter. The diluter was also

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Table 1. Chemical characteristics of test water

Characteristic	Unit	Mean* ± standard deviation	Range
Acidity	mg l^{-1} as $CaCO_3$	3±1.1	2–6
Alkalinity	mg l^{-1} as $CaCO_3$	42±4.3	39–46
Hardness	mg l^{-1} as $CaCO_3$	43±0.6	44–46
Dissolved oxygen	mg l^{-1}	9±2.2	5–13
pH	--	--	7–8

*Measured from all test tanks, one tank per week.

modified to deliver twice the volume of water to maintain previous flow rates. The chemical-metering apparatus was also adjusted so that five concentrations of Cr(VI) ranging from 0.10 to 1.56 mg Cr l^{-1} plus one control were maintained throughout the 8-month exposure.

Chemical

An aqueous solution of sodium dichromate, prepared from reagent grade $Na_2Cr_2O_7 \cdot 2H_2O$ (Fisher Scientific)*, was introduced to the exposure system via a chemical-metering apparatus described by Mount and Warner (1965). Test-water samples (20 ml) were acidified and composited from each exposure tank Monday through Friday. Mean weekly chromium concentrations and S.D. measured in each test tank were:

8-month brook trout exposure; <0.01 (Control), 0.01 ± 0, 0.02 ± 0.01, 0.4 ± 0.01, 0.10 ± 0.01, 0.20 ± 0.02;
8-month rainbow trout exposure; <0.01 (Control), 0.10 ± 0.02, 0.20 ± 0.03, 0.34 ± 0.1, 0.71 ± 0.04, 1.50 ± 0.05;
22-month brook trout exposure; <0.01 (Control), 0.35 ± 0.1, 0.76 ± 0.1, 1.56 ± 0.1, 3.10 ± 0.2, 6.37 ± 0.5.

Atomic absorption spectrometry was employed utilizing a Beckman model 979 atomic absorption spectrophotometer equipped with a Perkin-Elmer (10 cm) laminar flow burner. Measured amounts of sodium dichromate were added to control water in order to establish chromium standards which were aspirated into the atomic absorber to determine standard curves with which to plot the absorbance of the test-water samples. Nine bioassay water samples spiked with sodium dichromate showed that the above method of known additions gave a 98% mean recovery of chromium with a S.D. of 3.4. Chemical characteristics of the test water were measured according to standard methods of the American Public Health Association (1965) (Table 1).

Biological

Eight-month brook trout test. Brook trout eggs were stripped and fertilized from adult fish captured from the Cascade River in Cook County, Minnesota. All embryos were incubated in laboratory control water. The exposure was begun by distributing 500 eyed embryos to each of five chromium concentrations ranging from 0.01 to 0.20 mg Cr l^{-1} plus one control. Embryos were incubated in the test water by means of an apparatus described by McKim and Benoit (1971). After hatch, alevins were randomly thinned to 100 per concentration and transferred to growth chambers (McKim and Benoit, 1971). Two months after hatch all fish were moved to larger exposure tanks (30 × 40 × 183 cm) for the remaining 6 months. Fish were fed Oregon Moist trout food daily; the amount of food was calculated bi-monthly on the basis of water temperature and total body weight for each individual group of exposed fish. Total lengths were also measured on all fish at the end of the test.

Twenty-two-month brook trout test. This exposure was begun by randomly distributing eighty 1-week-old alevins to each of five chromium concentrations ranging from 0.35 to 6.37 mg Cr l^{-1} plus one control. Brook trout embryos collected for this test were obtained from the same previously described source and incubated in laboratory control water. Fish were fed the same food as during the previous exposure. Six months after the test was initiated control fish were randomly thinned to 20 to avoid overcrowding and to equalize numbers of fish between the control and surviving fish in the exposure tank so that growth rates could be compared throughout the experiment. Sex was determined on all fish 19 months after the test was initiated and were randomly thinned to two males and four females per tank. One spawning substrate described by McKim and Benoit (1971) was then placed in each exposure tank. During the fall spawning period each substrate was checked daily, and the eggs were removed, counted, and placed in oscillating incubation jars designed after those of Mount (1968). Two weeks after spawning ceased all substrates were removed. Adult fish were then killed, weighed, measured, and ovaries checked to determine which individuals had spawned. Methods utilized during embryo incubation and 3-month exposure of offspring were the same as those described by McKim and Benoit (1974). Two groups of embryos from control spawnings were also transferred, incubated, and hatched in water containing 0.76 and 1.56 mg Cr l^{-1}. This allowed direct comparisons of survival between acclimated alevins exposed as embryos and unacclimated 1-week-old alevins exposed at the start of this test.

Eight-month rainbow trout test. Rainbow trout embryos, obtained from the Cedar Island Fish Hatchery in Brule, Wisconsin, were incubated and hatched in laboratory control water. The exposure was begun by randomly distributing 40 1-week-old alevins to each of five chromium concentrations ranging from 0.1 to 1.5 mg Cr l^{-1} plus one control. Experimental test methods for this exposure were the same as those described for the 8-month brook trout exposure.

Acute toxicity tests. Duplicate 96-h acute toxicity tests were conducted at 12 ± 1°C with 5-month-old brook trout and 14-month-old rainbow trout from the same stock used in the chronic tests. Continuous flow tests using the proportional diluter with 10 fish per concentration were conducted according to methods described by the American Public Health Association (1965).

RESULTS

All 1-week-old rainbow trout and brook trout alevins exposed to 0.34 and 0.76 mg Cr l^{-1}, respectively, and higher concentrations died after the first 3 months of the tests (Figs. 1 and 2). Yolk-sac absorption was slower at these concentrations than in controls; the lethargic fish did not swim up to accept food and eventually starved to death. The death of all rainbow trout juveniles after 3 months' exposure to 0.34 mg Cr l^{-1} and of only 72% of the young brook trout during the first 3 months' exposure to 0.35 mg Cr l^{-1} indicated that rainbow trout were slightly more sensitive to chromium than brook trout.

Growth in weight was retarded at all test concentrations during the 8-month rainbow trout and 8-month brook trout exposures. At 0.20 mg Cr l^{-1} 8-month-old rainbow and brook trout weighed 30 and 20% less than controls, respectively. The 22-month exposure of surviving brook trout at

* Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

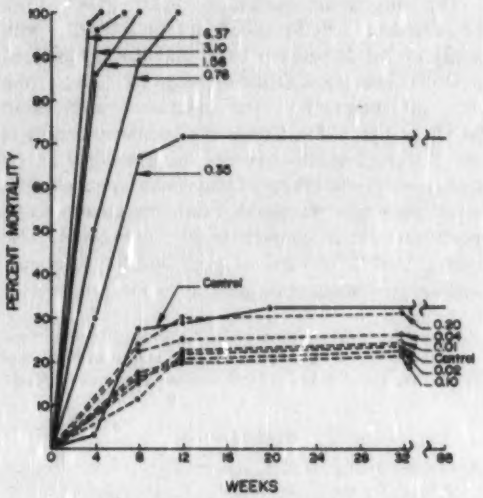

Fig. 1. Mortality of brook trout exposed to various concentrations of chromium (VI). Chromium concentrations are expressed in mg l^{-1}. Dashed lines: 8-month exposure (embryo through juvenile). Solid lines: 22-month exposure (alevin through adult).

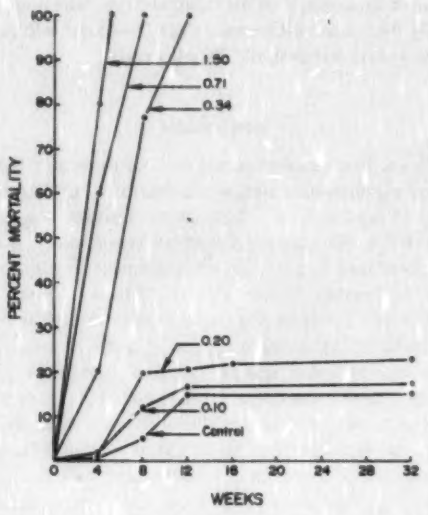

Fig. 2. Mortality of rainbow trout (alevin through juvenile) exposed to various concentrations of chromium (VI) for 8 months. Chromium concentrations are expressed in mg l^{-1}.

0.35 mg Cr l^{-1} showed that at 6 months of age the fish weighed 25% less than controls; however, these early effects on growth were temporary because weights of exposed brook trout, 12–22 months after hatch, varied only 10–12% from controls. The temporary effects on growth were, therefore, not used to calculate the MATC for brook trout.

A serious bacterial disease (*Aeromonas liquefaciens*) during the spawning phase of the 22-month brook trout exposure killed all but one female and one male in the test tank containing 0.35 mg Cr l^{-1}. The two remaining diseased fish at this concentration were able, however, to spawn successfully before dying; reproductive capacity of these fish compared favorably with that of the controls (Table 2). The added chromium stress during maturation and spawning probably increased the trout's susceptibility to this disease.

Ninetynine per cent of all spawned embryos hatched successfully at 0.35 mg Cr l^{-1}; however, after 3 months' exposure of offspring to this concentration, survival was 22% less than controls, and surviving fish were significantly smaller than control fish ($P = 0.05$; analysis of variance with t-test) (Table 2). The hatching success of control embryos transferred to 0.76 and 1.56 mg Cr l^{-1} averaged 98%; however, all the fish died within 3 months after hatch.

The mean 96-h LC_{50} of duplicate continuous-flow acute bioassays for rainbow trout and brook trout were 69 and 59 mg Cr l^{-1}, respectively. The higher

Table 2. Reproduction of brook trout exposed to various concentrations of chromium (VI) for 22 months; and 3-month survival and growth of offspring. Number in parenthesis indicates number of fish measured

	Measured chromium (mg l^{-1})			
Item	Control	0.35	0.76	1.56
Mean spawning per female	2.2	2.0	-*	-
Mean number of eggs spawned per female	338	205	-	-
Embryo hatchability (%)	96	99	98†	100†
Alevin-juvenile survival (%)	80	58	0	0
Alevin-juvenile growth				
Length (mm)	36.1±6.4‡ (40)	31.9±6.3§ (29)	-	-
Weight (mg)	53.4±24.2 (40)	38.9±20.4§ (29)		

*All fish died at this concentration and above.

†Control embryos transferred at spawning.

‡Mean and standard deviation.

§Significantly different than control (P=0.05; analysis of variance with t-test).

LC_{50} value established for rainbow trout was undoubtedly due to the difference in age (14-month-old rainbow versus 5-month-old brook trout).

DISCUSSION

In the three studies survival of rainbow and brook trout alevins was a sensitive indicator of Cr(VI) toxicity at 0.34 mg $Cr\,l^{-1}$ and above. Growth was also affected to various degrees at all chromium concentrations tested; however, measurements throughout the 22-month exposure of brook trout to 0.35 mg $Cr\,l^{-1}$ demonstrated that even though mortalities were extremely high and growth of surviving fish was retarded during the first 3 months after hatch, growth was comparable to controls throughout the remaining 19 months. Delayed growth could be used to measure permanent effects if the difference in size at that stage could be correlated with the fish's ability to feed on natural food and survive against predators or other environmental stresses not normally encountered in laboratory bioassays.

Olson and Foster (1956) exposed chinook salmon and rainbow trout alevins to Cr(VI) for 4 months and also found that survival and growth were affected. They concluded that a growth difference as small as 6% between control fish and fish exposed to 0.02 mg $Cr\,l^{-1}$ indicated that this concentration was unsafe for salmonids. However, if this study had been continued beyond 4 months, these small differences in growth probably could have disappeared and alevin survival would have then been used to measure the effects of chromium. On the basis of survival, their work demonstrated that chinook salmon and rainbow trout were adversely affected at 0.20 mg $Cr\,l^{-1}$, which agrees closely with the results of this report.

Recent work conducted by Sinley *et al.* (1974) and Spehar (1974) have shown that unacclimated rainbow trout and flagfish were approximately four times more sensitive to zinc than acclimated fish which were incubated and hatched in the same concentrations. This did not appear to be true for acclimated and unacclimated brook trout alevins exposed to chromium. Both groups of brook trout died within 3 months after hatch at 0.76 and 1.56 mg $Cr\,l^{-1}$; and only a small difference in survival was observed between the two groups exposed to 0.35 mg $Cr\,l^{-1}$ (i.e. survival at 3 months = 38% for acclimated offspring; 30% for unacclimated parental fish).

The chromium application factor (MATC/96-h LC_{50}) reported here for brook trout in soft water with a pH of 7–8 is between 0.003 and 0.006, based on a MATC between 0.20 and 0.35 mg $Cr\,l^{-1}$ and a 96-h LC_{50} of 59 mg $Cr\,l^{-1}$. The calculated brook trout MATC range is based upon the combined results of the 8- and 22-month-exposure using survival as the end point. If the effects of chromium upon rainbow trout are similar to brook trout, the concentration permitting alevin survival in this study would also be the MATC for rainbow trout and the chromium application factors would be similar for both species.

Acknowledgements—I thank Mr. R. Andrew for technical assistance, Mr. E. Leonard for chromium analyses, and Dr. J. McKim for his critical review of the manuscript.

REFERENCES

American Public Health Association (1965) *Standard Methods for the Examination of Water and Wastewater*, 12th edition, 769 pp. New York.

McKim J. M. & Benoit D. A. (1971) Effects of long term exposures to copper on survival, growth, and reproduction of brook trout, *Salvelinus fontinalis*. *J. Fish. Res. Bd Can.* **28**, 655–662.

McKim J. M. & Benoit D. A. (1974) Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout *Salvelinus fontinalis*. *J. Fish. Res. Bd Can.* **31**, 449–452.

Mount D. I. (1968) Chronic toxicity of copper to fathead minnows. *Water Res.* **2**, 215–223.

Mount D. I. & Brungs W. A. (1967) A simplified dosing apparatus for fish toxicology studies. *Water Res.* **1**, 21–29.

Mount D. I. & Stephan C. E. (1967) A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. *Trans. Am. Fish. Soc.* **96**, 185–193.

Mount D. I. & Warner R. E. (1965) A serial dilution apparatus for continuous delivery of various concentrations of materials in water. *U.S. Dep. Health Educ. Welfare Publ. Health Serv. Publ.* 999-WP-23. 16 p.

Olson P. A. & Foster R. F. (MS 1956) Effect of chronic exposure to sodium dichromate on young chinook salmon and rainbow trout. *Battelle Pac. Northwest Lab.* HW-41500: 34–47.

Pickering Q. H. & Henderson C. (1966) The acute toxicity of some heavy metals to different species of warmwater fishes. *Int. J. Air Wat. Pollut.* **10**, 453–463.

Sinley J. R., Goettl J. P. & Davies P. H. (1974) The effects of zinc on rainbow trout in hard and soft water. *Bull. Environ. Contam. Toxicol.* **12**, 193–201.

Spehar R. L. (1974) Cadmium and zinc toxicity to *Jordanella floridae*: Effects on growth, reproduction, survival, and behavior. MS Thesis, University of Minnesota, Duluth, Minn. 66 p.